Journal of Applied Organometallic Chemistry

2024, Volume 4, Issue 2

Original Article

Novel Mefenamic Acid Analogs Featuring 4-Thiazolidinone Moiety: Design, Synthesis, *In Silico* Modeling and Biological Evaluation

Arefeh Badrzadeh Shekarab[a, †]| Niloofar Gharahchoulou[a, †]| Seyed Sam Banisadr[a]| Maryam Bayanati[b]| Mohammad Mahboubi-Rabbani[a]| Shima H.M.E Ketabforoosh[c]| Homa Azizian[d]| Ali Almasirad[a,*]

[a]*Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*
[b]*Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[c]*Department of Medicinal Chemistry, School of Pharmacy, Alborz University of Medical Sciences, Karaj, Iran*
[d]*Department of Medicinal Chemistry, School of Pharmacy, Iran University of Medical Science, Tehran, Iran*

Use your device to scan and read the article online

Citation A. Badrzadeh Shekarab, N. Gharahchoulou, S. S. Banisadr, M. Bayanati, M. Mahboubi-Rabbani, S. H.M.E Ketabforoosh, H. Azizian, A. Almasirad, **Novel Mefenamic Acid Analogs Featuring 4-Thiazolidinone Moiety: Design, Synthesis, *In Silico* Modeling and Biological Evaluation.** *J. Appl. Organomet. Chem.*, **2024**, *4*(2), 119-134.

https://doi.org/10.48309/JAOC.2024.440607.1162

Article info:
Submitted: 02 February 2024
Revised: 07 March 2024
Accepted: 10 March 2024

ID: JAOC-2401-1162
Checked for Plagiarism: Yes
Language Editor Checked: Yes

Keywords:
Thiazolidinone, Molecular docking, Mefenamic acid, Cyclooxygenase, NSAIDs, Synthesis

ABSTRACT

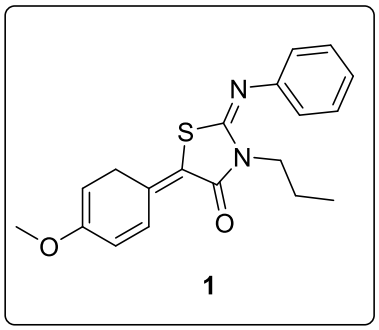
In this work, a novel series of mefenamic acid analogs were developed and synthesized with the goal of developing a lead chemical that has anti-inflammatory efficacy and avoids the adverse effects of NSAIDs. Molecular docking analysis was performed by recruiting the ligands, COX-1 and COX-2 to identify the best-fitted molecule using AutoDock software. Afterwards, the compounds were synthesized and analyzed. To assess the drug's efficacy, the compounds were subjected to *in vivo* analgesic and anti-inflammatory experiments. Most of synthesized ligands have greater binding free energy than mefenamic acid on COX-1. When compared to the positive control, the compounds 2-(2,3-dimethylphenylamino)-*N*-(2-(3,5-di-tert-butyl-4-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzamide**,** 2-(2,3-dimethylphenylamino)-*N*-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)benzamide**,** 2-(2, 3-dimethylphenylamino)-*N*-(4-oxo-2-p-tolylthiazolidin-3-yl) benzamide and 2-(2,3-dimethylphenylamino)-*N*-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl) benzamide**,** 2-(2,3-dimethylphenylamino)-*N*-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)benzamide demonstrated a larger or comparable proportion of analgesic and anti-inflammatory action respectively. Furthermore, the selected compounds "2-(2,3-dimethylphenylamino)-*N*-(2-(3,5-di-tert-butyl-4-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzamide", and "2-(2,3-dimethylphenylamino)-*N*-(4-oxo-2-p-tolylthiazolidin-3-yl) benzamide" seemed to have the least ulcerogenic activity. These findings show that some of the newly created mefenamic acid analogs may be selected as lead compounds due to their significant biological properties without ulcerogenic activity.

***Corresponding Authors: Ali Almasirad** (*almasirad.a@iaups.ac.ir*)

Introduction

Non-steroidal anti-inflammatory medications (NSAIDs) are a class of pharmacologically-active compounds used to suppress pain and inflammation [1] . These chemicals inhibit the two cyclooxygenase isoforms (COX-1 and COX-2) in a non-selective manner [2]. COX-1 inhibition disrupted homeostatic function, but COX-2 inhibition resulted in anti-inflammatory action [3-5]. Inhibiting COX causes an increase in leukotrienes, which are produced via the 5-lipoxygenase pathway, resulting in a variety of negative effects such as gastrointestinal damage and other inflammatory events [6,7]. Many publications exist on the various chemical structures of dual COX/5-LO inhibitors, which provide a better method for generating novel effective drugs with the fewest adverse effects [8-14]. The thiazolidine-4-one derivatives demonstrated stereoselective anti-inflammatory and analgesic activities with fewer gastrointestinal side effects, as well as inhibition of COX-2 isoforms just like compound **1** (Scheme 1) [15-18].

As a result of the foregoing observation, we used a hybridization technique to create a novel series of mefenamic acid derivatives by inserting a thiazolidinone ring. All of the target compounds have been tested for anti-inflammatory, analgesic, and ulcerogenic properties. The results of this work are reported as a continuation of our previous studies with the aim of designing and synthesizing COX inhibitors as analgesic compounds [19-21].



1

Scheme 1. The chemical structure of compound **1**

Experimental

Chemistry

The starting materials and other corresponding reagents were obtained from Merck chemical company (Tehran, Iran), and used without further purification. An electrothermal IA 9300 capillary melting-point apparatus (Ontario, Canada) was used to measure the uncorrected melting points. IR spectra were captured using a Shimadzu FT-IR 8400 apparatus. ^{1}H-NMR spectra were obtained on a Bruker FT-NMR (AC-400 MHZ). Tetramethylsilane (TMS) was employed as an internal standard. The Mass spectra were also recorded on a Finnigan Mat TSQ-70 spectrometer at 70 eV. Thin layer chromatography (TLC) was also utilized to investigate the progression of the chemical reactions along with the reactivity and purity of the produced materials.

*General synthesis of (E)-N'-benzylidene-2-((2,3-dimethylphenyl)amino) benzohydrazide analogs (**5a-5n**)*

The main approach for preparing mefenamic acid hydrazone derivatives (Scheme 2) followed the previously established procedures [6]. In this study, we synthesized some new hydrazone derivatives using the corresponding aromatic aldehydes. Compounds **5c**, **5e**, **5f**, and **5n** were newly-synthesized derivatives.

*(E)-2-(2, 3-Dimethylphenylamino)-N'-(2-chlorobenzylidene) benzohydrazide (**5c**)*

Yield 95%; m.p. = 212-213 °C. FT-IR (KBr): cm^{-1}, 3320 (NH), 3200 (NH), 1633 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 9.85 (bs, 1H, NH), 8.02 (s, 1H, NH), 8.05 (s, 1H, N=CH), 7.84 (d, J = 8.0 Hz, 1H, aromatic C-H), 7.51-7.43 (m, 1H, aromatic C-H), 7.42-7.31 (m, 2H, aromatic C-H), 7.26 (t, 1H, aromatic C-H), 7.13-7.04 (m, 3H, aromatic C-H), 6.91 (d, J = 7.2 Hz, 1H, aromatic C-H), 6.88 (d, J = 7.6 Hz, 1H, aromatic C-H), 6.76 (t, 1H, aromatic C-H), 2.28 (s, 3H, CH_3), 2.16 (s, 3H, CH_3); Mass: m/z (%), 379 (M^++2, 10), 377 (M^+, 30), 224 (100), 209 (32), 194 (11), 180 (25).

*(E)-2-(2,3-Dimethylphenylamino)-N'-(2,6-dichlorobenzylidene)benzohydrazide (**5e**)*

Yield 94%; m.p. = 246-247 °C. FT-IR (KBr): cm^{-1}, 3313 (NH), 3232 (NH), and 1636 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 12.1 (s, 1H, NH), 9.24 (bs, 1H, NH), 8.62 (s, 1H, =CH), 7.77 (d, J= 8.4 Hz, 1H, aromatic C-H), 7.56 (d, J = 8.0 Hz, 2H, aromatic C-H), 7.44 (t, 1H, aromatic C-H), 7.31 (t, 1H, aromatic C-H), 7.09-7.07 (m, 2H, aromatic C-H), 6.99 (d, J = 4.8 Hz, 1H, aromatic C-H), 6.85-6.78 (m, 2H, aromatic C-H), 2.28 (s, 3H, CH_3), and 2.12 (s, 3H, CH_3); Mass: m/z (%), 415 (M+4,4), 413 (M+2, 20), 411 (M^+,30), 224 (100), 209 (31), 194 (18), and 180 (31).

*(E)-2-(2, 3-Dimethylphenylamino)-N'-(3,5-di-tert-butyl-4-hydroxybenzylidene)benzohydrazide (**5f**)*

Yield 85%; m.p. = 270-271 °C. FT-IR (KBr): cm^{-1}, 3628 (OH, non-bonded), 3450 (OH, bonded), 3328 (NH), 3210 (NH), and 1635 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 11.65 (s, 1H, NH), 9.21 (s, 1H, NH), 8.35 (s, 1H, =CH), 7.74 (d, J=7.2 Hz, 1H, aromatic C-H), 7.47-7.44 (m, 3H, aromatic C-H) 7.29 (t, 1H, aromatic C-H), 7.09 (m. 2H, aromatic C-H), 6.96 (m. 1H, aromatic C-H), 6.83 (d, J = 8.0 Hz, 1H, aromatic C-H), 6.79 (t, 1H, aromatic C-H), 2.28 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), and 1.42 (s, 18H, t-Bu-CH_3); Mass: m/z (%), 471 (M+,15), 248 (16), 224 (100), 209 (29), 194 (10), and 180 (17).

*(E)-2-(2, 3-Dimethylphenylamino)-N'-((thiophen-2-yl)methylene) benzohydrazide (**5n**)*

Yield 96%; m.p. = 230-232 °C. FT-IR (KBR): cm^{-1}, 3312, 3204 (NH), and 1627 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 11.8 (s, 1H, NH) 9.22 (s, 1H, NH), 8.62 (s, 1H, N=CH), 7.70 (d, J = 8.0 Hz, 1H, aromatic C-H), 7.67 (d, J = 4.8 Hz, 1H, thiophene C-H), 7.47 (d, J=2.8 Hz, 1H, thiophene C-H), 7.29 (t, 1H, aromatic C-H), 7.11 (m, 3H, aromatic C-H), 6.95 (d, J=5.8 Hz, 1H, thiophene C-H), 6.84 (d, J = 8.0 Hz, 1H, aromatic C-H) 6.79 (t, 1H, aromatic C-H), 2.28 (s, 3H, CH_3), and 2.12 (s,3H, CH_3); Mass: m/z (%), 349 (M^+, 51), 224 (100), 209 (33), 194 (12), and 180 (27).

*General synthesis of 2-(2,3-dimethylphenylamino)-N-(2-(aryl)-4-oxothiazolidine-3-yl) benzamide analogs (**6a-6n**)*

To a solution of (**5a-5n**) (2.68 mmol) in dry toluene (20 ml), thioglycolic acid (0.37 ml, 5.36 mmol) and anhydrous $ZnCl_2$ were added, and the reaction mixture was refluxed for 24 hours using a “Dean-stark” water separator. TLC was used to confirm the accomplishment of the reaction, after which the solvent was withdrawn, and the residue was neutralized using a 10 % $(NH_4)_2CO_3$ solution. The crude product was washed with water, dried, and recrystallized from ethanol.

*2-(2,3-Dimethylphenylamino)-N-(4-oxo-2-phenylthiazolidin-3-yl) benzamide (**6a**)*

Yield 64%; m.p. = 217-218 °C. FT-IR (KBr): cm^{-1}, 3328 (NH), 3169 (NH), 1695 (C=O), and 1670 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.65 (s, 1H, NH), 9.05 (s, 1H, NH), 7.51 (d, J = 6.0 Hz, 2H, aromatic C-H), 7.45 (d, J = 8.0 Hz, 1H, aromatic C-H), 7.38 (m, 3H, aromatic C-H), 7.25 (t, 1H, aromatic C-H), 7.09-7.07 (m, 2H, aromatic C-H), 6.99-6.98 (m, 1H, aromatic C-H), 6.74 (d, J = 8.0 Hz, 1H, aromatic C-H), and 6.05 (t, 1H, aromatic C-H); Mass: m/z (%), 417 (M^+, 26), 224 (100), 209 (31), 194 (10), and 180 (21). Anal. Calcd. For $C_{24}H_{23}N_3O_2S$: C, 69.04; H, 5.55; N, 10.06; Found: C, 69.10; H, 5.54; and N, 10.05.

*2-(2,3-Dimethylphenylamino)-N-(2-(4-bromophenyl)-4-oxothiazolidin-3-yl) benzamide (**6b**)*

Yield 81%; m.p. = 225-227 °C. FT-IR (KBr): cm^{-1}, 3321 (NH), 3185 (NH), 1680 (C=O), and 1650 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.75 (s, 1H, NH), 9.05 (s, 1H, N-H), 7.58 (d, J= 8.4 Hz, 2H, aromatic C-H), 7.48 (d, J = 8.4 Hz, 1H, aromatic C-H), 7.47 (d, J = 8.4 Hz, 2H, aromatic C-H), 7.25 (t, 1H, aromatic C-H), 7.07 (m, 2H, aromatic C-H), 6.98 (m, 1H, aromatic C-H), 6.74 (d, J = 8.4 Hz, 1H, aromatic C-H), 6.67 (t, 1H, aromatic C-H), 5.95 (s. 1H, Ar-CH), 3.96, 3.82 (2d, J= 16.8 Hz, 2H, CH_2- s), 2.28 (s, 3H, CH_3), and 2.08 (s,3H,CH_3); Mass: m/z (%), 497 (M^++2, 22), 495 (M^+, 22), 224 (100), 209 (29), 194 (15),

and 180(25). Anal. Calcd. For $C_{24}H_{22}BrN_3O_2S$: C, 58.07; H, 4.47; N, 8.46; Found: C, 58.12; H, 4.45; and N, 8.48.

*2-(2,3-Dimethylphenylamino)-N-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl) benzamide (**6c**)*

Yield 92%; m.p. = 218-219 °C. FT-IR (KBr): cm^{-1}, 3312 (NH), 3178 (NH), 1687 (C=O), and 1663 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.82 (s, 1H, NH), 9.04 (s, 1H, NH), 7.75 (d, J = 5.2 Hz, 1H, aromatic C-H), 7.52 (d, J = 7.6 Hz, 1H, aromatic C-H), 7.48-7.39 (m, 3H, aromatic C-H), 7.29-7.23 (m, 1H, aromatic C-H), 7.09-7.07 (m, 2H, aromatic C-H), 7.09-7.07 (m, 1H, aromatic C-H), 6.74 (d, J = 9.2 Hz, 1H, aromatic C-H), 6.68 (t, 1H, aromatic C-H), 6.28 (s, 1H, Ar-CH), 3.95, 3.83 (2d, J = 15.6 Hz, S-CH_2), 2.28 (s, 3H, CH_3), and 2.08 (s, 3H, CH_3); Mass: m/z (%), 453 (M^++2, 6), 451 (M^+, 18), 224 (100), 209 (26), 194 (9), 180 (19). Anal. Calcd. For $C_{24}H_{22}ClN_3O_2S$: C, 63.78; H, 4.91; N, 9.30; Found: C, 63.73; H, 4.92; and N, 9.29.

*2-(2, 3-Dimethylphenylamino)-N-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl) benzamide (**6d**)*

Yield 86%; m.p. = 198-200 °C. FT-IR (KBr): cm^{-1}, 3167, 3333 (NH), 1690 (C=O), and 1648 (C=O). ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.75 (s, 1H, NH), 9.04 (s, 1H, NH), 7.55 (d, J = 8.4 H, 2H, aromatic C-H) 7.44 (d, J= 8.4 Hz, 1H, aromatic C-H), 7.43 (d, J = 8.4 Hz, 2H, aromatic C-H), 7.25 (t, 1H, aromatic C-H), 7.07 (m, 2H, aromatic C-H), 6.98 (m, 1H, aromatic C-H), 6.74 (d, J = 8.0 Hz, 1H, aromatic C-H), 6.64 (t, 1H, aromatic C-H), 5.96 (s, 1H, Ar-CH), 3.96, 3.82 (2d, J = 15.2 Hz, 2H, S-CH_2), 2. 27 (S, 3H, CH_3), and 2.07 (s, 3H, CH_3) Mass: m/z (%), 453 (M^++2, 4), 451 (M^+, 12), 224 (100), 209 (27), 180 (16), 166 (13), 111 (12), 85 (24), 71 (31), and 57 (40). Anal. Calcd. For $C_{24}H_{22}ClN_3O_2S$: C, 63.78; H, 4.91; N, 9.30; Found: C, 63.75; H, 4.89; and N, 9.29.

*2-(2,3-Dimethylphenylamino)-N-(2-(2,6-dichlorophenyl)-4-oxothiazolidin-3-yl)benzamide (**6e**)*

Yield 80%; m.p. = 232-233 °C. FT-IR (KBr): cm^{-1}, 3324 (NH), 3215 (NH), 1699 (C=O), and 1659 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.8 (s, 1H, NH), 8.85 (s, 1H, NH), 7.57 (d, J = 7.6 Hz, 1H, aromatic C-H), 7.49-7.42 (m, 3H, aromatic C-H), 7.26 (t, 1H, aromatic C-H), 7.09-7.07 (m, 2H, aromatic C-H), 6.98 (m, 1H, aromatic C-H), 6.8 (s, 1H, Ar-CH), 6.74 (d, J = 8.4 Hz, 1H, aromatic C-H), 6.66 (t, 1H, aromatic C-H), 3.95, 3.90 (2d, J = 16.0 Hz, 2H, S-CH_2), 2.28 (s, 3H, CH_3), and 2.09 (s, 3H, CH_3); Mass: m/z (%), 489 (M+4, 1.5), 487 (M+2, 9), 485 (M+,13), 224 (100), 209 (30), 194 (11), 180 (21). Anal. Calcd. For $C_{24}H_{21}Cl_2N_3O_2S$: C, 59.26; H, 4.35; N, 8.64; Found: C, 59.28; H, 4.34; and N, 8.65.

*2-(2, 3-Dimethylphenylamino)-N-(2-(3,5-di-tert-butyl-4-hydroxyphenyl)-4-oxothiazolidin-3-yl) benzamide (**6f**)*

Yield 70%; m.p. = 228-229 °C. FT-IR (KBr): cm-^{1}, 3600 (OH-non-bonded), 3400 (OH, bonded), 3332 (NH), 1697 (C=O), and 1646 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.5 (s, 1H, NH), 9.05 (s, 1H, NH), 7.42 (d, J = 8.4 Hz, 1H, aromatic C-H), 7.23 (s, 2H, aromatic C-H), 7.09-7.05 (m, 3H, aromatic C-H), 6.96 (m, 1H, aromatic C-H), 6.76 (d, J = 8.4 Hz, 1H, aromatic C-H), 6.65 (t, 1H, aromatic C-H), 5.89 (s, 1H, Ar-CH), 3.84, 3.80 (2d, J = 16.0 Hz, 2H, s-CH_2), 2.27 (s, 3H, CH_3), 2.08 (s, 3H, CH_3) 1.33 (s, 18H, t-Bu-CH_3). Mass: m/z (%), 545 (M^+, 6), 240 (22), 224 (100), 209 (23), and 180 (14). Anal. Calcd. For $C_{32}H_{39}N_3O_3S$: C, 70.43; H, 7.20; N, 7.70; Found: C, 70.40; H, 7.22; and N, 7.69.

*2-(2, 3-Dimethylphenylamino)-N-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)benzamide (**6g**)*

Yield 65%; m.p. = 209-210 °C. FT-IR (KBr): cm^{-1} 3324 (NH), 3162 (NH), 1693 (C=O), and 1676 (C=O); ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.65 (s, 1H, NH), 9.05 (s, 1H, NH), 7.59-7.55 (M, 2H, aromatic C-H), 7.45 (d, J = 8.4 Hz, 1H, aromatic C-H), 7.27-7.19 (m, 3H, aromatic C-H), 7.11-7.07 (M, 2H, aromatic C-H), 7.02-6.98 (m, 1H, aromatic C-H), 6.74 (d, J = 8.8 Hz, 1H, aromatic C-H), 6.67-6.64 (t, 1H, aromatic C-H), 5.96 (s, 1H, Ar-CH), 3.95, 3.82 (2d, J = 16.0 Hz, S-CH_2), 2.28 (s, 3H, CH_3), and 2.07 (s, 3H, CH_3). Mass: m/z (%), 435 (M^+, 20), 224 (100), 209 (18), 194 (11), 180 (20), 81 (52), 69 (98), and

57 (61). Anal. Calcd. For $C_{24}H_{22}FN_3O_2S$: C, 66.19; H, 5.09; N, 9.65; Found: C, 66.22; H, 5.11; and N, 9.66.

*2-(2, 3-Dimethylphenylamino)-N-(4-oxo-2-p-tolylthiazolidin-3-yl) benzamide (**6h**)*

Yield 55%; m.p. = 226-227 °C. FT-IR (KBr): cm^{-1}, 3325 (NH), 3168 (NH), 1676 (C=O), and 1663 (C=O). ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.65 (s, 1H, NH), 9.0 (s, 1H, NH), 7.44 (d, J = 8.4 Hz, 1H, aromatic C-H), 7.38 (d, J = 8.0 Hz, 2H, aromatic C-H), 7.25 (t, 1H, aromatic C-H), 7.18 (d, J=7.6 Hz, 2H, aromatic C-H), 7.09-7.07 (m, 2H, aromatic C-H), 6.98 (m 1H, aromatic C-H), 6.74 (d, J = 8.4 Hz, 1H, aromatic C-H), 6.65 (t, 1H, aromatic C-H), 5.92 (s, 1H, Ar-CH), 3.91, 3.81 (2d, J = 16.4 Hz, 2H, SCH_2), 2.28 (s, 6H, CH_3), and 2.08 (s, 3H, CH_3). Mass: m/z (%), 431 (M+, 25), 224 (100), 209 (34), 194 (12), 180 (23). Anal. Calcd. For $C_{25}H_{25}N_3O_2S$: C, 69.58; H, 5.84; N, 9.74; Found: C, 69.55; H, 5.85; and N, 9.75.

*2-(2, 3-Dimethylphenylamino)-N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl) benzamide (**6i**)*

Yield 55%; m.p. = 209-210 °C. FT-IR (KBr): cm^{-1}, 3338 (NH), 3178 (NH), 1686 (C=O), and 1677 (C=O). ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.65 (s, 1H, NH), 9.03 (s, 1H, NH), 7.44-7.23 (m, 3H, aromatic C-H), 7.25 (t, 1H, aromatic), 7.09-7.07 (m, 2H, aromatic C-H), 6.99-6.97 (m, 1H, aromatic C-H), 6.92 (d, J= 8.8 Hz, 2H, aromatic C-H), 6.74 (d, J = 8.4 Hz, 1H, aromatic C-H), 6.65 (m, 1H, aromatic C-H), 5.96 (s, 1H, Ar-CH), 3.90, 3.81 (2d, J = 16.4 Hz, 2H, S-CH_2), 3.74 (s, 3H, OCH_3), 2.28 (s, 3H, CH_3), and 2.08 (s, 3H, CH_3). Mass: m/z (%), 447 (M$^+$, 16), 240 (6), 224 (100), 209 (26), 194 (9), 180 (16). Anal. Calcd. For $C_{25}H_{25}N_3O_3S$: C, 67.09; H, 5.63; N, 9.39; Found: C, 67.20; H, 5.64; and N, 9.37.

*2-(2, 3-Dimethylphenylamino)-N-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)benzamide (**6j**)*

Yield 62%; m.p. = 198-199 °C. FT-IR (KBr): cm^{-1}, 3285 (NH), 1694 (C=O), 1667 (C=O), 1528, and 1348 (NO_2). ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 8.23 (d, J = 8.4 Hz, 2H, aromatic C-H), 7.82 (d, J = 8.4 Hz, 2H, aromatic C-H), 7.48(d, J=6.8Hz, 1H, aromatic C-H), 7.25(t, 1H, aromatic C-H), 7.08d-7.06, (m, 2H, aromatic C-H), 6.98-6.97(m, 1H, aromatic C-H), 6.76(d, J=8.4 Hz, 1H, aromatic C-H), 6.67(t, 1H, aromatic C-H), 6.10 (s, 1H, Ar-CH), 4.04, 3.85(2d, J=16.0Hz, 2H, S-CH2), 2.28(s, 3H, CH3), 2.07(s, 3H, CH3). Mass: m/z (%), 462(M$^+$,23), 224(100), 209(32), 194(11), and 180(21). Anal. Calcd. For $C_{24}H_{22}N_4O_4S$: C, 62.32; H, 4.79; N, 12.11; Found: C, 62.30; H, 4.78; and N, 12.12.

*2-(2,3-Dimethylphenylamino)-N-(4-oxo-2-(pyridin-2-yl)thiazolidin-3-yl) benzamide (**6k**)*

Yield 42%; m.p. = 230-233 °C. FT-IR (KBr): cm^{-1}, 3311, 3233 (NH), 1703 (C=0), and 1660 (C=0). ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.85 (s.1H, NH), 9.12 (s, 1H, NH), 8.55 (d, J=4.5Hz, 1H, pyridine C-H), 7.88 (t, 1H, pyridine C-H), 7.64 (d, J=7.5 Hz, 1H, aromatic C-H), 7.50 (d, J=8.0 Hz, 1H, pyridine C-H), 7.38 (t, 1H, pyridine C-H), 7.26 (t, 1H, aromatic C-H), 7.12-6.97 (m, 2H, aromatic C-H) 6.97 (m, 1H, aromatic C-H), 6.95 (d, J=8.4 Hz, 1H, aromatic C-H), 6.65 (t, 1H, aromatic C-H), 5.92 (s. 1H, CH-Ar), 3.96, 3.79 (d, J=16.0 Hz, 2H, S-CH_2), 2.28 (s, 3H, CH_3), and 2.08 (s, 3H, CH_3). Mass: m/z (%), 418 (M$^+$, 22), 224 (100), 209 (28), 194 (12), and 180 (31). Anal. Calcd. For $C_{23}H_{22}N_4O_2S$: C, 66.01; H, 5.30; N, 13.39; Found: C, 66.06; H, 5.32; and N, 13.40.

*2-(2,3-Dimethylphenylamino)-N-(4-oxo-2-(pyridin-3-yl)thiazolidin-3-yl)benzamide (**6l**)*

Yield 26%; m.p. = 200-201 °C. FT-IR (KBr): cm^{-1}, 3320 (NH), 3294 (NH), 1723 (C=O), and 1642 (C=O). ^{1}H-NMR (400 MHz, d^6-DMSO): ppm, 10.75 (s, 1 H, NH), 9. 06 (s, 1H, NH). 8.70 (s, 1H, pyridine C-H), 8.55 (d, J= 4.0 Hz, 1H, pyridine C-H), 7.96 (d, J= 7.6 Hz, 1H, Pyridine C-H), 7.44 (m, 2H, aromatic C-H, pyridine), 7.25 (t, 1H, aromatic C-H), 7.07 (m, 2H, aromatic C-H), 6.98 (m, 1H, aromatic C-H), 6.74 (d, J = 8.0 Hz, 1H, aromatic C-H), 6.65 (t, 1H, aromatic C-H), 6.01 (s, 1H, CH-Ar) 4.02, 3.84 (2d, J = 16.0 Hz, 2H, S-CH_2), 2.28 (s, 3H, CH_3), and 2.08 (s, 3H, CH_3). Mass: m/z (%), 418 (M$^+$, 4), 279 (18), 224 (33), 207 (32) 194 (22), 180 (19), 111 (44), 85 (62), 71 (80), and 57 (100). Anal. Calcd. For

$C_{23}H_{22}N_4O_2S$: C, 66.01; H, 5.30; N, 13.39; Found: C, 66.05; H, 5.30; and N, 13.41.

*2-(2,3-Dimethylphenylamino)-N-(4-oxo-2-(pyridin-4-yl)thiazolidin-3-yl)benzamide (**6m**)*

Yield 31%; m.p. = 213-216 °C. FT-IR (KBr):cm^{-1}, 1667 (C=O), and 3322 (NH); ^{1}H-NMR: (400 MHZ, d^6-DMSO): ppm, 10.8 (s, 1H, NH), 9.12 (s, 1H, NH), 8.6 (d, J= 4.5 Hz, 2H, Pyridine C-H), 7.55 (d, J = 4 Hz, 2H, pyridine C-H), 7.49 (d, J=8 Hz, 1H, aromatic C-H), 7.4-6.67 (m, 6H, aromatic C-H), 5.97 (s, 1H, Py-CH), 3.97, 3.85 (2d, J = 16.0 Hz, 2H, S-CH_2), 2.37(s, 3H, CH_3), and 2.08 (s, 3H, CH_3). Mass: m/z (%), 418 (M^+, 36), 224 (100), 209 (35), 194 (33), 71 (27), and 57 (38). Anal. Calcd. For $C_{23}H_{22}N_4O_2S$: C, 66.01; H, 5.30; N, 13.39; Found: C, 66.03; H, 5.31; and N, 13.41.

*2-(2,3-Dimethylphenylamino)-N-(4-oxo-2-(thiophen-2-yl)thiazolidin-3-yl)benzamide (**6n**)*

Yield 46%; m.p. = 222-224 °C. FT-IR (KBr): cm^{-1}, 3342, 3224 (NH), 1702 (C=O), and 1663 (C=O); ^{1}H-NMR: (400 MHz, d^6-DMSO): ppm, 10.76 (s, 1H, NH), 9.2 (s, 1H, NH), 7.65 (d, J = 5.3 Hz, 1H, thiophene), 7.52 (d, J = 7.2 Hz, 1H, aromatic C-H), 7.27 (m, 2H, aromatic C-H, thiophene), 7.09 (m, 2H, aromatic C-H), 6.99 (m, 2H, aromatic C-H, thiophene), 6.75 (d, J = 8.0 Hz, 1H, aromatic C-H), 6.68 (t, 1H, aromatic C-H), 6.23 (s, 1H, Ar-CH), 3.82, 3.85 (2d, J = 15.5 Hz, 2H, S-CH_2), 2.28 (s, 3H, CH_3), and 2.09 (s, 3H, CH_3). Mass: m/z (%), 423 (M^+, 27), 224 (100%), 209 (32), 194 (13), and 180 (26). Anal. Calcd. For $C_{22}H_{21}N_3O_2S_2$: C, 62.39; H, 5.00; N, 9.92; Found: C, 62.52; H, 5.02; and N, 9.93.

Pharmacological evaluation

Male NMRI mice weighing 20-25 g were used to test synthetic compounds' analgesic activity. Male rats weighing 150 g were also recruited to assess the anti-inflammatory and ulcerogenic actions; animals were purchased from the Pasteur Institute of Iran (Karaj, Iran). Except during the studies, the animals had free access to food and water. The animals were kept in a constant temperature (22± 2 °C) environment with a 12 h light/dark cycle. The study procedure was approved by the Islamic Azad University Tehran Medical Sciences animal ethics committee.

Analgesic activity

In vivo, analgesic efficacy was determined using an acetic acid (% 0.6; 0.1 ml/10g) writhing test in mice mice [7, 22]. The test compounds were given to the animals at a dose of 30 mg/kg, followed by an intraperitoneal injection of acetic acid solution 30 minutes later. Analgesic activity was measured by counting the number of writhing movements made by each animal immediately after an acetic acid injection, as well as 30 minutes. The anti-nociceptive impact was indicated as a percentage decrease in writhing number as compared to the negative control group.

Anti-inflammatory activity

The carrageenan-induced rat paw edema simulation model was used to evaluate the anti-inflammatory activity of the newly-synthesized compounds [6,23,24]. A 0.1 ml solution of carrageenan in saline (Sigma-Aldrich, Dorset, UK) was administered through the injection into the palmar surface of each animal's right hind paw. The compounds and standard medication were given intraperitoneally one hour before the carrageenan solution injection. Dial calipers were utilized for determining paw thickness shortly before carrageenan injection, as well as at regular intervals over the next five hours. Anti-inflammatory activity was measured as the percentage of edema inhibition compared to the standard group.

Ulcerogenic activity

Acute ulcerogenicity was determined using the method described before by Cioli *et al.* [25]. The rats were divided into groups of six. The test compounds and the reference medication (Indomethacin) were given orally at dosages of 105 and 210 mg/kg, respectively. The control group was given 1 ml of a 0.5 CMC solution. The animals were fasted for 24 hours before the test compounds were administered, with free access to water. The rats were fed a regular meal for 17 hours following oral administration of newly

discovered compounds and the positive control. The stomach was cracked open in tandem with its greater curvature and cleaned with distilled water and normal saline. The mucosal tissue damage was examined employing a magnifying lens while the subsequent grading system: 0.5 for redness, 1.0 for spot ulcers, 1.5 for hemorrhagic streaks, 2.0 for ulcers bigger than three but less than five, and 3.0 for ulcers greater than five [21,26].

Statistical analysis

The data are shown as the average ± SEM for n animals. The results were statistically assessed via one-way ANOVA, accompanied by the Tukey multicomparison test [27]. Statistically noteworthy variations between groups of experiments were defined as $p < 0.05$.

Docking studies

Docking analyses against COX-1 (PDB code: 3kk6) were conducted using the active compound **6h**. Topspin 3.5.7 software was used to examine the binding mode of COX-1 inhibitory action. Every one of the compounds was created and then optimized utilizing HyperChem 8.0 program [28]. To build the protein architecture for docking, we utilized AutoDock tools 1.5.6 from the MGL Tools package [29, 30]. Docking was carried out by the Autodock VINA [31]. All water molecules and co-crystallized ligand were eliminated from the protein 3D crystal structure. Polar hydrogens were incorporated, and non-polar hydrogens were merged prior to the addition of the Kollman unified atom charge and atom type parameter. Grid map dimensions were established around the active site (20×20×20).

Results and Discussion

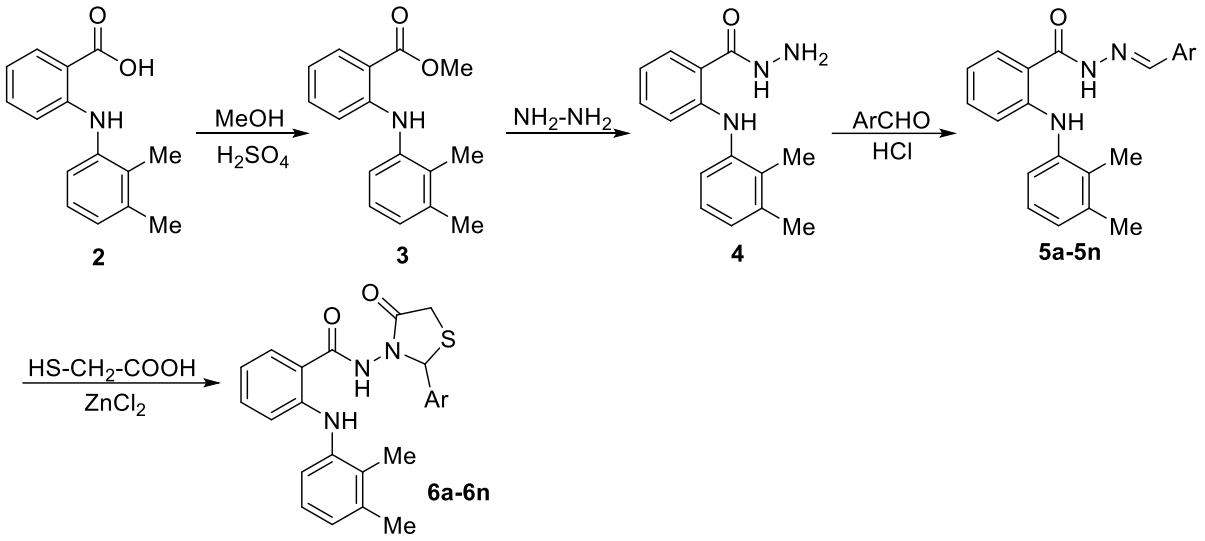
Synthesis

The synthetic route of final compounds is outlined in Scheme 2. The starting compound, mefenamic acid (**2**), reacted with anhydrous methanol and H_2SO_4 98% as the catalyst to give methyl 2-(2,3-dimethylphenylamino)benzoate (**3**), which then treated with hydrazine hydrate to afford 2-(2,3-dimethylphenylamino) benzohydrazide (**4**) [6,20,32,33]. Further reaction with different substituted aromatic or heteroaromatic aldehydes yielded the corresponding 2-(2,3-dimethylphenylamino)-*N*'-(aryl-heteroaryl) benzohydrazide analogs **5a**-**5n**. The compounds underwent cycloaddition with thioglycolic acid in presence of anhydrous $Zncl_2$ to get 2-(2,3-dimethylphenylamino)-*N*-(2-(aryl)-4-oxothiazolidine-3-yl) benzamide target derivatives **6a**-**6n**.

All of the newly-developed compounds **6a**-**6n** were evaluated for their analgesic activity. Some of the molecules, including **6d**, **6f**, **6g**, **6h**, **6j**, **6m**, and **6n** which were confirmed to be the most potent analgesics, via the writhing test were assayed for anti-inflammatory activity. Moreover, compounds **6f** and **6h** which were significantly active in the writhing test were screened for their acute ulcerogenicity.

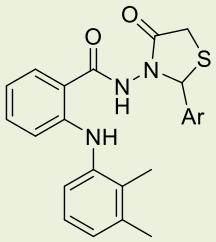
Evaluation of the analgesic activity

Table 1 summarizes the anti-nociceptive effect of all recently discovered thiazolidine-4-one derivatives. Except for compounds **6b** and **6l**, all of the novel molecules reduced the writhing response significantly when compared to the control. Among the newly developed analogs, certain substances inhibited abdominal constriction more effectively than mefenamic acid. Thus, substituting 4-thiazolidinone for the acidic component of mefenamic acid may result in high analgesic action. As a result, it may be hypothesized that the thiazolidinone ring and its substituents may interact with the COX binding site in various ways. The hypothesis was justified by the outcomes of the docking study, as discussed later in more details. The bioisosteric replacement of phenyl with thienyl gives a more potent compound **6n**. The existence of electron attracting groups on the para position of the phenyl ring is better than ortho, and both electron withdrawing or electron donating moieties on the para position give more active derivatives.



Scheme 2. Preparation of target compounds **6a-6n**

Table 1. Analgesic activity of compounds **6a-6n** (Writhing test)



Compound	Ar	Constriction No. (mean ± SEM)	Inhibition (%)	Relative activity	*P*-value
6a	phenyl	14.00 ± 3.114	56.48	0.95	< 0.01
6b	4-bromophenyl	20.60 ± 2.768	35.97	0.60	> 0.05
6c	2-chlorophenyl	11.60 ± 3.600	63.94	1.07	< 0.001
6d	4-chlorophenyl	6.00 ± 1.414	81.35	1.37	<.0.001
6e	2,6-dichlorophenyl	11.20 ± 1.530	65.18	1.09	< 0.001
6f	3,5-di-tert-butyl-4-hydroxyphenyl	4.00 ± 1.049	87.57	1.47	< 0.001
6g	4-flourophenyl	3.40 ±1.435	89.43	1.50	< 0.001
6h	4-methylphenyl	3.00 ± 0.837	90.67	1.52	< 0.001
6i	4-methoxyphenyl	9.80 ± 1.934	69.54	1.17	< 0.001
6j	4-nitrophenyl	7.60 ± 0.678	76.38	1.28	< 0.001
6k	2-pyridyl	14.40 ± 4.812	55.24	0.93	< 0.01
6l	3-pyridyl	21.20 ± 6.398	34.10	0.57	> 0.05
6m	4-pyridyl	6.60 ± 1.965	79.48	1.33	< 0.001
6n	2-thienyl	5.40 ± 2.821	83.21	1.40	< 0.001
Mefenamic acid	-	13.0 ± 2.098	59.59	1.00	< 0.001
Control	-	32.17 ± 1.759	-	-	-

Table 2. The anti-inflammatory activities of compounds **6d, 6f, 6g, 6h, 6j, 6m,** and **6n** (Carrageenan-induced rat paw edema)

Compound	Ar	Time (h)	Thickness variation (mm)	Inhibition (%)	P-value
6d	4-Chlorophenyl	1	1.274 ± 0.214	53.02	< 0.01
		2	1.966 ± 0.185	49.97	< 0.01
		3	2.402 ± 0.283	48.60	< 0.01
		4	2.164 ± 0.275	52.56	< 0.001
		5	1.674 ± 0.293	56.02	< 0.01
6f	3,5-Di-tert-butyl-4-hydroxyphenyl	1	1.468 ± 0.282	45.87	< 0.05
		2	2.498 ± 0.337	36.44	> 0.05
		3	3.284 ± 0.455	29.74	> 0.05
		4	3.308 ± 0.317	27.49	> 0.05
		5	3.038 ± 0.311	20.18	> 0.05
6g	4-Flourophenyl	1	1.872 ± 0.343	30.97	> 0.05
		2	2.510 ± 0.250	36.13	> 0.05
		3	2.714 ± 0.227	41.93	< 0.05
		4	2.774 ± 0.185	39.19	< 0.01
		5	2.108 ± 0.338	44.61	< 0.05
6h	4-Methylphenyl	1	1.904 ± 0.170	29.79	> 0.05
		2	2.804 ± 0.371	28.65	> 0.05
		3	3.472 ± 0.463	25.72	> 0.05
		4	3.316 ± 0.371	27.31	> 0.05
		5	2.654 ± 0.458	30.27	> 0.05
6j	4-Nithrophenyl	1	1.664 ± 0.102	38.64	> 0.05
		2	2.116 ± 0.311	46.16	< 0.05
		3	2.504 ± 0.388	46.43	< 0.05
		4	2.708 ± 0.369	40.64	< 0.01
		5	2.326 ± 0.314	38.89	> 0.05
6m	4-Pyridil	1	1.568 ± 0.186	42.18	< 0.05
		2	2.826 ± 0.433	28.09	> 0.05
		3	4.300 ± 0.336	8.00	> 0.05
		4	4.416 ± 0.190	3.20	> 0.05
		5	3.826 ± 0.282	-0.52	> 0.05
6n	2-Thienyl	1	1.246 ± 0.391	54.05	< 0.01
		2	2.262 ± 0.524	42.44	< 0.05
		3	3.382 ± 0.647	27.64	> 0.05
		4	3.500 ± 0.464	23.28	> 0.05
		5	2.990 ± 0.485	21.44	> 0.05
Indomethacin	-	1	1.098 ± 0.173	59.44	< 0.001
		2	1.604 ± 0.234	59.18	< 0.001
		3	1.734 ± 0.236	62.90	< 0.001
		4	1.368 ± 0.224	70.01	< 0.001
		5	1.104 ± 0.209	70.99	< 0.001
Control	-	1	2.712 ± 0.066	-	-
		2	3.930 ± 0.318	-	-
		3	4.674 ± 0.366	-	-
		4	4.562 ± 0.355	-	-
		5	3.806 ± 0.185	-	-

The existence of electron attracting groups on the para position of the phenyl ring is better than ortho, and both electron withdrawing or electron donating moieties on the para position give more active derivatives.

Evaluation of the anti-inflammatory activity

The anti-inflammatory effects of compounds **6d**, **6f**, **6g**, **6h**, **6j**, **6m**, and **6n** are reported in Table 2. Compound **6d**, which has a 4-chlorophenyl group at the second position of the thiazolidinone ring, had the highest anti-inflammatory efficacy even after 5 hours (56.02%). The anti-inflammation action of the compound **6n** (Inh%), which has a thienyl group on the thiazolidinone ring, was proven to be 54.05, and 42.44% during the first 2 hours, respectively, but drastically declined after 2 hours. The anti-inflammatory action was reduced when the *ρ*-chlorophenyl motif was changed into the "3, 5-di-tert-butyl-4-hydroxyphenyl, *ρ*-fluorophenyl, *ρ*-nitrophenyl, and 4-pyridyl moieties, as found in compounds **6f**, **6g**, **6j**, and **6m**. Compound **6h**, which included 4-methylphenyl, was ineffective for 5 hours. In conclusion, the anti-inflammatory effect was connected to the type of the thiazolidinone ring substituents. The 4-chlorophenyl group inhibited edema more than the other compounds, which might be attributed to its hydrophobic binding capabilities. Replacement of the phenyl ring with heterocyclic bioisosteres (thienyl or pyridyl) has a deteriorative effect on the activity. The Electron acceptor groups like F, Cl, and NO_2 are preferred to methyl as an electron-donating moiety.

Evaluation of the ulcerogenic activity

In terms of ulcerogenicity, two of the compounds with the strongest analgesic effect were chosen to be evaluated. The acute ulcerogenicity of compounds **6f** and **6h** is outlined in Table 3. The newly-developed chemicals indicated a significant reduction in ulcerogenicity with a severity index ranging from 0.300 ± 0.123 to 0.200 ± 0.123, while the reference drug, indomethacin, produced a high severity index (1.667 ± 0.333). A massive reduction in ulcerogenic effects was identified for the compound **6f**, possessing 3, 5-di-tert-butyl-4-hydroxyphenyl group at the thiazolidinone ring.

Docking studies

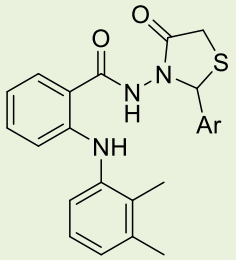
A docking analysis was used to evaluate the orientation of the very efficient anti-inflammatory analog (6h) into the COX-1 binding site (Figure 1). The docking scores for all of the newly-designed analogs were also summarized in Table 4. Accordingly, the binding score for the most active agents 6f and 6h were the highest ones, in accordance with the results of the analgesic evaluation. Interestingly, they found also as the least selective COX inhibitors. According to this molecular modeling, 6h bound with the main binding site, inserting the dimethyl phenyl group substituent into the hydrophobic pocket found in COX-1. The phenyl ring with two adjacent methyl substituents was orientated towards a hydrophobic pocket at the top of the COX-1 main binding site composed of Ile523 and Tyr385. The benzoyl moiety also interacted with the lipophilic backbone, consisting of Ile345, Leu534, and Met113.

According to our theory, the carbonyl was located near Ser530, the aspirin acetylation site. In this regard, the O-atom of the carbonyl moiety in the thiazolidinone was roughly 3.4 A away from the OH of Tyr348, the other carbonyl was close to the OH of Ser530 (distance = 3.7 A), potentially contributing to covalent acetylation of COX-1 Ser530 (Figure 1).

QSAR studies

The quantitative structure-activity relationship (QSAR) links a compound's molecular structure to its biological effects, consequently built models aid to predict the activity of newly-designed molecules [34-36]. As a result, we may use QSAR models to create novel structures. To determine if there is a link between the analgesic and anti-inflammatory effects of thiazolidine-4-one derivatives and their physiochemical parameters, a QSAR investigation employing a range of molecular

Table 3. Acute ulcerogenic activity of compounds **6f**, and **6h**



Compound	Ar	Dose (mg/kg)	Ulcerogenic activity (Severity index ± SEM)	*P*-value
6f	3,5-Di-tert-butyl-4-Hydroxyphenyl	210	0.200 ± 0.123	> 0.05
6h	4-Methylphenyl	210	0.300 ± 0.123	> 0.05
Indomethacin	-	210	1.667 ± 0.333	< 0.001
Control	-	-	0.00 ± 0.00	-

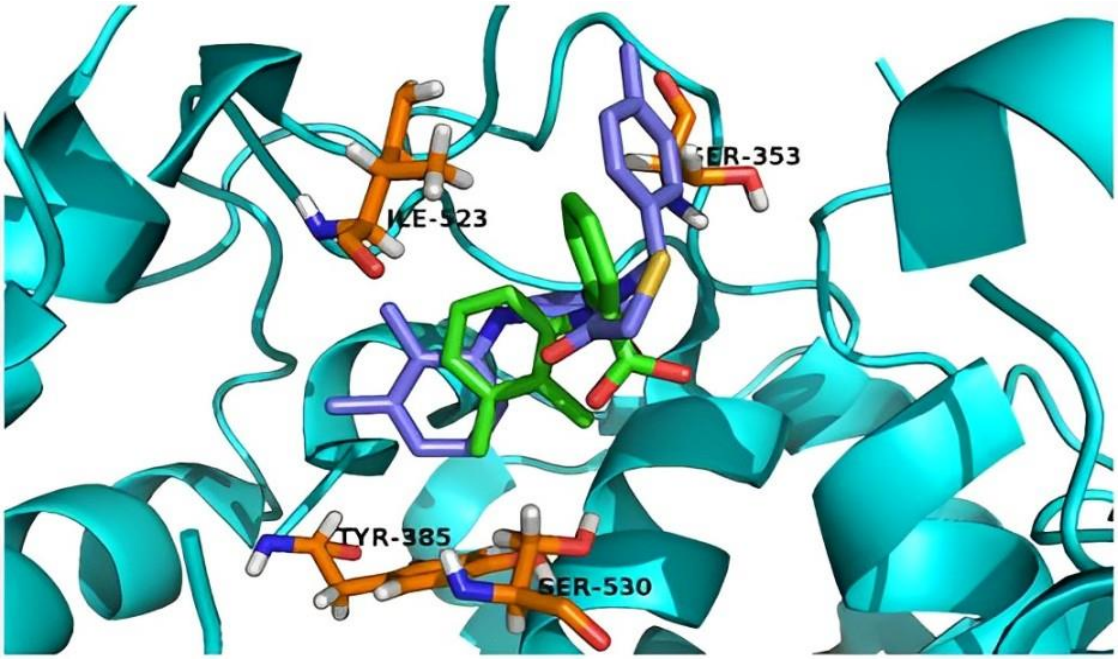


Figure 1. Three dimensional superimposed representation of mefenamic acid and **6h** with the active site of COX-1 (PDB code: 3kk6)

descriptors was conducted in this work. The analgesic/anti-inflammatory activity was expressed as % inhibition [37]. Hyperchem software was used to create the chemical structures of the compounds (Hypercube Inc, USA). Because the predicted values of various electronic descriptors are depend on the three-dimensional molecular geometry, the molecules' 3D geometry was adjusted using the AM1 semi-empirical approach. Electronic and physiochemical descriptors were obtained by Hyperchem. Dragon software was used to compute topological descriptors. To obtain the optimal multi-linear regression Equation, we employed stepwise selection methods of SPSS software's multiple linear regression (MLR) subdivision subdivision [38, 39]. Since collinearity of descriptors distorts MLR results, correlation analysis was performed using MATLAB software. Each of the co-linear descriptors exhibiting the most robust connection with biological activity was kept.

Some prominent parameters, including the root mean square error (RMSE), correlation coefficient (r^2), correlation coefficient for cross-validation significance (q2), standard error of regression (SE), and significant level were utilized to verify the regression Equation (*p*-value) [40]. The following Equation was obtained for the analgesic activity of the newly-developed compounds:

% Inhibition = 15.429 (± 4.566) L_3m + 25.380 (± 13.475)

N=14, R^2=0.488, RMSE = 14.4964, SE = 13.77792, q^2 = 14.4964, F = 11.419

The resulting Equation for the anti-inflammatory effect of the newly discovered compounds is displayed within every hour of edema separately:

For the 1st hour, % Inhibition= 241.447 (± 65.951) E_3v-24.22 (± 18.229)

N=7, R^2 = 0.728, RMScv = 6.4747, SE = 5.53424, q^2 = 0.5760, F = 13.403

For the 2nd hour: % Inhibition = 196.470 (±24.837) Dm -28.166 (±8.451)

N=7, R^2 = 0.926, RMSE = 2.8818, SE = 2.49497, q^2 = 0.8830, F = 62.574

For the 3rd hour: % Inhibition = 90.453 (± 17.371) H_3m - 105.529 (± 26.626)

N=7, R^2=0.844, RMSE = 7.7943, SE = 6.16264, q^2 = 0.7047, F = 27.113

For the 4th hour: % Inhibition = 48.492 (± 15.973) Qpos-139.322 (± 56.079)

N = 7, R^2 = 0.648, RMSE = 13.3333, SE = 10.20873, q^2 = 0.3495, F = 9.217

For the 5th hour; PI= 63.651 (± 14.266) Qpos-192.815 (± 50.088)

N = 7, R^2 = 0.799, RMSE = 12.0088, SE = 9.11806, q^2 = 0.6037, F = 19.906

The second hour edema findings revealed the best association by computed descriptors among such Equations. Dm is a WHIM and a 3D molecular descriptor. The WHIM descriptors include information on the size, shape, symmetry, and atom distribution of a molecule's structure. D is the total density atom inside a molecule, whereas Dm denotes the total accessibility index/weighted by mass. Actually, Dm denotes the presence of mass concentration at the terminus of a molecule [41,42]. Since Dm has a positive impact in second hour Equation, increasing the mass of the group on the thiazolidine-4-one ring improves the anti-inflammatory action of compounds. The predicted values for "% Inhibition" of the newly-developed compounds using second hour Equation are listed in **Table 5**. The correlations between experimental and predicted activity levels are shown in **Figure 2**.

Table 4. Docking scores for newly-designed analogs **6a-6n**

Compound	COX-1	COX-2
6a	-8.6	-5.3
6b	-8.0	-2.8
6c	-8.3	-5.6
6d	-8.7	-6.6
6e	-7.5	-4.5
6f	-9.1	-1.6
6g	-8.2	-4.9
6h	-8.6	-4.6
6i	-8.7	-6.7
6j	-7.4	-6.6
6k	-7.7	-7.0
6l	-7.9	-5.3
6m	-8.2	-4.5
6n	-8.3	-5.0
Celecoxib	-9.6	-10.9
Mefenamic acid	-8.2	-8.2

Table 5. The chemical descriptor values, experimental and anticipated levels of the studied substances' anti-inflammatory activities (the 2nd hour)

Compound	Dm	Experimental activity (% Inhibition)	Predicted activity (% Inhibition)
6d	0.40	49.97	50.62
6f	0.34	36.44	38.24
6g	0.32	36.13	34.31
6h	0.29	28.65	28.61
6j	0.36	46.16	41.97
6m	0.29	28.09	29.60
6n	0.37	42.44	44.53

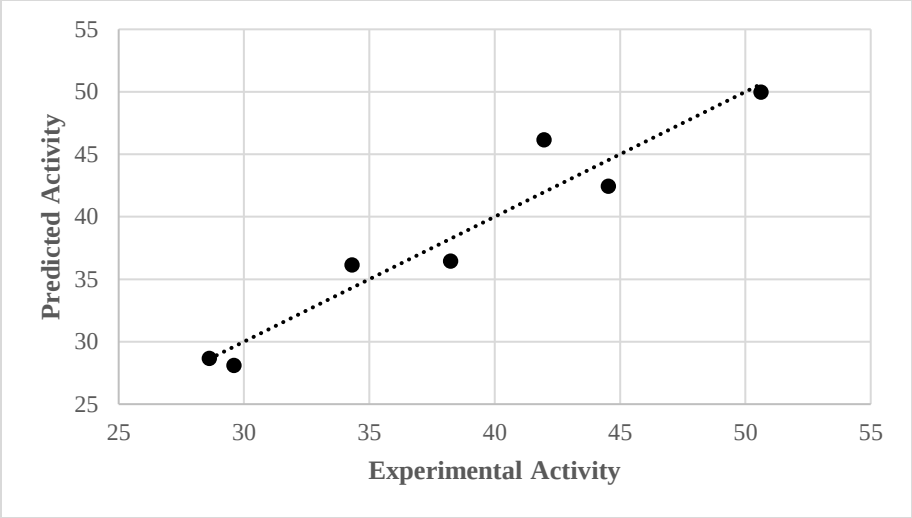


Figure 2. Experimental vs. predicted anti-inflammatory activity represented as % inhibition

Conclusion

In summary, a novel series of mefenamic acid compounds containing 4-thiazolidinone were produced and tested for anti-inflammatory efficacy in vivo. All of the novel analogs demonstrated good to exceptional anti-inflammatory efficacy. Compounds **6g** and **6h** were shown to have the most significant analgesic action. The 2-position of the 4-thiazolidinone ring was shared by all active molecules. The inclusion of an aryl ring in the 2 position significantly increased inhibitory action. The docking investigation revealed that the activity is dependent on the establishment of a hydrogen bond between the carbonyl and the crucial enzyme residue Ser530, as well as a hydrophobic interaction between the dimethyl phenyl moiety and Tyr385.

Acknowledgments

The authors are grateful to the late Professor Abbas Shafiee for his invaluable assistance in making this study feasible.

Conflict of Interests

The authors disclose no conflicts of interest.

Orcid

Maryam Bayanati
https://orcid.org/0000-0002-1225-7538

Mohammad Mahboubi-Rabbani
https://orcid.org/0000-0003-3512-7688

Shima H.M.E Ketabforoosh
https://orcid.org/0000-0001-8817-3050

Homa Azizian
https://orcid.org/0000-0003-0827-3727

Ali Almasirad
https://orcid.org/0000-0001-9192-7888

References

[1]. M. Abdel-Tawab, H. Zettl, M. Schubert-Zsilavecz, Nonsteroidal anti-inflammatory drugs: a critical review on current concepts applied to reduce gastrointestinal toxicity, *Current Medicinal Chemistry*, **2009**, *16*, 2042-2063. [Crossref], [Google Scholar], [Publisher]

[2]. H.A. Al-Haboubi, I.J. Zeitlin, Re-appraisal of the role of histamine in carrageenan-induced paw oedema, *European Journal of Pharmacology*, **1983**, *88*, 169-176. [Crossref], [Google Scholar], [Publisher]

[3]. A. Almasirad, M. Tajik, D. Bakhtiari, A. Shafiee, M. Abdollahi, M.J. Zamani, R. Khorasani, H. Esmaily, Synthesis and analgesic activity of *N*-arylhydrazone derivatives of mefenamic acid, *Journal of Pharmaceutical Sciences*, **2005**, *8*, 419-425. [Crossref], [Google Scholar]

[4]. A. Almasirad, A. Shafiee, M. Abdollahi, A. Noeparast, N. Shahrokhinejad, N. Vousooghi, S.A. Tabatabai, R. Khorasani, Synthesis and analgesic activity of new 1, 3, 4-oxadiazoles and 1, 2, 4-triazoles, *Medicinal Chemistry Research*, **2011**, *20*, 435-442. [Crossref], [Google Scholar], [Publisher]

[5]. G. Sharma, S.B. Sharma, Synthetic Impatienol analogues as potential cyclooxygenase-2 inhibitors: A preliminary study, *Journal of Applied Organometallic Chemistry*, **2021**, *1*, 66-75. [Crossref], [Google Scholar], [Publisher]

[6]. A. Almasirad, S. Samiee-Sadr, A. Shafiee, Synthesis and antimycobacterial activity of 2-(Phenylthio) benzoylarylhydrazone derivatives, *Iranian Journal of Pharmaceutical Research*, **2011**, *10*, 727-731. [Google Scholar], [Publisher]

[7]. A. Almasirad, Z. Mousavi, M. Tajik, M.J. Assarzadeh, A. Shafiee, Synthesis, analgesic and anti-inflammatory activities of new methyl-imidazolyl-1, 3, 4-oxadiazoles and 1, 2, 4-triazoles, *Daru Journal of Pharmaceutical Sciences*, **2014**, *22*, 1-8. [Crossref], [Google Scholar], [Publisher]

[8]. A. Almasirad, P.S.V. Sani, Z. Mousavi, G.B. Fard, T. Anvari, M. Farhadi, M. Vosooghi, H. Azizian, Novel thiazolotriazolone derivatives: design, synthesis, in silico investigation, analgesic and anti-inflammatory activity, *Chemistry Select*, **2022**, *7*, e202103228. [Crossref], [Google Scholar], [Publisher]

[9]. M. Amir, S.A. Javed, H. Kumar, Design and synthesis of 3-[3-(substituted phenyl)-4-piperidin-1-ylmethyl/-4-morpholin-4-ylmethyl-4, 5-dihydro-isoxazol-5-yl]-1 *H*-indoles as potent anti-inflammatory agents, *Medicinal Chemistry Research*, **2010**, *19*, 299-310. [Crossref], [Google Scholar], [Publisher]

[10]. S. Bindu, S. Mazumder, U. Bandyopadhyay, Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective, *Biochemical Pharmacology*, 2020, *180*, 114147. [Crossref], [Google Scholar], [Publisher]

[11]. A.M. Brown, A new software for carrying out one-way ANOVA post hoc tests, *Computer Methods and Programs in Biomedicine*, **2005**, *79*, 89-95. [Crossref], [Google Scholar], [Publisher]

[12]. V. Cioli, S. Putzolu, V. Rossi, P.S. Barcellona, C. Corradino, The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats, *Toxicology and Applied Pharmacology*, **1979**, *50*, 283-289. [Crossref], [Google Scholar], [Publisher]

[13]. M. Fernández, J. Caballero, QSAR modeling of matrix metalloproteinase inhibition by *N*-hydroxy-*α*-phenylsulfonylacetamide derivatives, *Bioorganic & Medicinal Chemistry*, **2007**, *15*, 6298-6310. [Crossref], [Google Scholar], [Publisher]

[14]. J.M. Figueiredo, C. de A Câmara, E.G. Amarante, A.L.P. Miranda, F.M. Santos, C.R. Rodrigues, C.A.M. Fraga, E.J. Barreiro, Design and synthesis of novel potent antinociceptive agents: methyl-imidazolyl *N*-acylhydrazone derivatives, *Bioorganic & Medicinal Chemistry*, **2000**, *8*, 2243-2248. [Crossref], [Google Scholar], [Publisher]

[15]. L.C. Gerstenfeld, M. Thiede, K. Seibert, C. Mielke, D. Phippard, B. Svagr, D. Cullinane, T.A. Einhorn, Differential inhibition of fracture healing by non-selective and cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs, *Journal of Orthopaedic Research*, **2003**, *21*, 670-675. [Crossref], [Google Scholar], [Publisher]

[16]. A.A. Geronikaki, A.A. Lagunin, D.I. Hadjipavlou-Litina, P.T. Eleftheriou, D.A. Filimonov, V.V. Poroikov, I. Alam, A.K. Saxena, Computer-aided discovery of anti-inflammatory thiazolidinones with dual cyclooxygenase/lipoxygenase inhibition, *Journal of Medicinal Chemistry*, **2008**, *51*, 1601-1609. [Crossref], [Google Scholar], [Publisher]

[17]. M.M. Hanna, New pyrimido [5, 4-e] pyrrolo [1, 2-c] pyrimidines: Synthesis, 2D-QSAR, anti-inflammatory, analgesic and ulcerogenicity studies, *European Journal of Medicinal Chemistry*, **2012**, *55*, 12-22. [Crossref], [Google Scholar], [Publisher]

[18]. D. Kadam, S. Patil, D. Mammen, S. Kadam, V. More, In silico molecular docking against-KIT Tyrosine Kinase and ADME studies of 4-thiazolidinone derivatives, *Journal of Applied Organometallic Chemistry*, **2023**, *3*, 13-27. [Crossref], [Google Scholar], [Publisher]

[19]. M.N. Koopaei, M.J. Assarzadeh, A. Almasirad, S.F. Ghasemi-Niri, M. Amini, A. Kebriaeezadeh, N.N. Koopaei, M. Ghadimi, A. Tabei, Synthesis and analgesic activity of novel hydrazide and hydrazine derivatives, *Iranian Journal of Pharmaceutical Research*, **2013**, *12*, 721-727. [Crossref], [Google Scholar], [Publisher]

[20]. M. Khoshneviszadeh, N. Edraki, R. Miri, B. Hemmateenejad, Exploring QSAR for substituted 2-sulfonyl-phenyl-indol derivatives as potent and selective COX-2 inhibitors using different chemometrics tools, *Chemical Biology & Drug Design*, **2008**, *72*, 564-574. [Crossref], [Google Scholar], [Publisher]

[21]. S.H.E. Ketabforoosh, M. Amini, M. Vosooghi, A. Shafiee, E. Azizi, F. Kobarfard, Synthesis, evaluation of anticancer activity and QSAR study of heterocyclic esters of caffeic acid, *Iranian Journal of Pharmaceutical Research*, **2013**, *12*, 705. [Google Scholar], [Publisher]

[22]. D. Laxmi, S. Priyadarshy, HyperChem 6.03, *Biotech Software & Internet Report: The Computer Software Journal for Scientists*, **2002**, *3*, 5-9. [Crossref], [Google Scholar], [Publisher]

[23]. K. Meirer, D. Steinhilber, E. Proschak, Inhibitors of the arachidonic acid cascade: interfering with multiple pathways, *Basic & Clinical Pharmacology & Toxicology*, **2014**, *114*, 83-91. [Crossref], [Google Scholar], [Publisher]

[24]. M. Mahboubi-Rabbani, A. Zarghi, Dual human carbonic anhydrase/cyclooxygenase-2 inhibitors: A promising approach for cancer treatment, *Anti-Cancer Agents in Medicinal Chemistry*, **2021**, *21*, 2163-2180. [Crossref], [Google Scholar], [Publisher]

[25]. M. Mahboubi-Rabbani, M. Abbasi, A. Zarghi, Natural-derived COX-2 inhibitors as anticancer drugs: A review of their structural diversity and mechanism of action, *Anti-Cancer Agents in Medicinal Chemistry*, **2023**, *23*, 15-36. [Crossref], [Google Scholar], [Publisher]

[26]. M. Mahboubi-Rabbani, A. Zarghi, Selective COX-2 inhibitors as anticancer agents: A patent review (2014-2018), *Expert Opinion on Therapeutic Patents*, **2019**, *29*, 1-21. [Crossref], [Google Scholar], [Publisher]

[27]. M. Mahboubi-Rabbani, A. Zarghi, Lipoxygenase inhibitors as cancer chemopreventives: Discovery, recent developments and future perspectives, *Current Medicinal Chemistry*, **2021**, *28*, 1143-1175. [Crossref], [Google Scholar], [Publisher]

[28]. G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, *Journal of Computational Chemistry*, **1998**, *19*, 1639-1662. [Crossref], [Google Scholar], [Publisher]

[29]. T. Previtera, M. Vigorita, M. Basile, G. Fenech, A. Trovato, F. Occhiuto, M. Monforte, R. Barbera, 3, 3′-di (1, 3-thiazolidine-4-one) system. V. Synthesis and pharmacological properties of 3, 3′(1, 2-ethanediyl) *bis*-(2-heteroaryl-1, 3-thiazolidine-4-one) derivatives, *European Journal of Medicinal Chemistry*, **1990**, *25*, 569-579. [Crossref], [Google Scholar], [Publisher]

[30]. H. Al-Madhagi, Empowering therapeutic strategies against methicillin-resistant staphylococcus aureus riboswitch: Unveiling the potential of small molecules and antisense oligonucleotides through in silico analysis, *Advanced Journal of Chemistry, Section A,* **2024**, *7*, 15-26. [Crossref], [Google Scholar], [Publisher]

[31]. A.A. Rad, M. Sheikhha, R. Hosseini, S.A. Tabatabaic, A. Shafiee, Synthesis and Morphine Enhancement Activity of *N*-[5-(2-phenoxyphenyl)-1, 3, 4-oxadiazole-2-yl]-*N*′-phenylurea derivatives, *Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry*, **2004**, *337*, 193-200. [Crossref], [Google Scholar], [Publisher]

[32]. L.S. Simon, Role and regulation of cyclooxygenase-2 during inflammation, *The American Journal of Medicine*, **1999**, *106*, 37S-42S. [Crossref], [Google Scholar], [Publisher]

[33]. R. Todeschini, P. Gramatica, The WHIM theory: New 3D molecular descriptors for QSAR in environmental modelling, *SAR and QSAR in Environmental Research*, **1997**, *7*, 89-115. [Crossref], [Google Scholar], [Publisher]

[34]. R. Todeschini, P. Gramatica, SD-modelling and prediction by WHIM descriptors. Part 5. Theory development and chemical meaning of WHIM descriptors, *Quantitative Structure-Activity Relationships*, **1997**, *16*, 113-119. [Crossref], [Google Scholar], [Publisher]

[35]. A. John, A. Ebune, I. Aderemi, A. Moyosore, G. Idah, Theoretical investigation and design of novel anti-proliferative agents against hepatocellular carcinoma from benzimidazole-chalcone derivatives, *Advanced Journal of Chemistry, Section A,* **2023**, *6*, 92-104. [Crossref], [Google Scholar], [Publisher]

[36]. K. Aminu, A. Uzairu, S. Abechi, G. Adamu, A. Umar, A search for novel antidiabetic agents using ligand-based drug design and molecular docking studies employing human intestinal maltase-glucoamylase as model enzyme, *Advanced Journal of Chemistry, Section A*, **2023**, *6*, 155-171. [Crossref], [Google Scholar], [Publisher]

[37]. O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, *Journal of Computational Chemistry*, **2010**, *31*, 455-461. [Crossref], [Google Scholar], [Publisher]

[38]. M. Vigorita, R. Ottanà, F. Monforte, R. Maccari, M. Monforte, A. Trovato, M. Taviano, N. Miceli, G. De Luca, S. Alcaro, Chiral 3, 3′-(1, 2-ethanediyl)-*bis* [2-(3, 4-dimethoxyphenyl)-4-thiazolidinones] with anti-inflammatory activity. Part 11: Evaluation of COX-2 selectivity and modelling, *Bioorganic & Medicinal Chemistry*, **2003**, *11*, 999-1006. [Crossref], [Google Scholar], [Publisher]

[39]. F. Ugbe, G. Shallangwa, A. Uzairu, I. Abdulkadir, A 2-D QSAR modeling, molecular docking study and design of 2-arylbenzimidazole derivatives as novel leishmania inhibitors: A molecular dynamics study, *Advanced Journal of Chemistry, Section A*, **2023**, *6*, 50-64. [Crossref], [Google Scholar], [Publisher]

[40]. A. Verma, S.K. Saraf, 4-Thiazolidinone–A biologically active scaffold, *European Journal of Medicinal Chemistry*, **2008**, *43*, 897-905. [Crossref], [Google Scholar], [Publisher]

[41]. J.L. Wallace, Distribution and expression of cyclooxygenase (COX) isoenzymes, their physiological roles, and the categorization of nonsteroidal anti-inflammatory drugs (NSAIDs), *The American Journal of Medicine*, **1999**, *107*, 11-16. [Crossref], [Google Scholar], [Publisher]

[42]. B. Whittle, The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics, *British Journal of Pharmacology and Chemotherapy*, **1964**, *22*, 246-253. [Crossref], [Google Scholar], [Publisher]